

CLAIMS

1. The use of a protein called sulfiredoxin (Srx),
5 which comprises at least one catalytic site having
the following motif: FXGCHR, with X = G or S, for
catalyzing the reduction of peroxyredoxins (Prxs)
in their superoxide form Prx-Cys_p-SO₂H
10 (peroxyredoxin cysteine sulfinic acid) to a thiol
derivative (SH).
2. The use as claimed in claim 1, characterized in
that said sulfiredoxin is a sulfiredoxin of a
15 microorganism, a plant or a higher organism, which
generally comprises between 80 and 170 amino acids
and at least the catalytic site having the
following motif: FXGCHR, with X = G or S, and
having the following percentage identities and
similarities:
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 - yeast/human: 32% identity and 67% similarity
 - yeast/plants: 23% identity and 39% similarity
 - yeast/mouse: 31% identity and 51% similarity
 - yeast/fungi: 80% identity and 90% similarity.
- 25 3. The use as claimed in claim 1 or claim 2,
characterized in that said sulfiredoxin is in
particular selected from proteins whose sequences
correspond, respectively, to the sequences SEQ ID
No. 1 to 10.
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4. An isolated peptide corresponding to the catalytic
site of Srx, as defined in claims 1 to 3,
characterized in that it is defined by the
following sequence; FXGCHR, with X = S.
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5. A medicinal product, characterized in that it
comprises an effective amount of a protein defined
by a sequence selected from the group consisting

of the sequences SEQ ID No. 1-3 and 5-10, and, optionally at least one pharmaceutically acceptable excipient.

- 5 6. The use of a protein as defined in claims 1 to 3, for preparing an antioxidanting medicinal product for use in the treatment of cancers, neurodegenerative disorders and neuromuscular diseases, in which a fault in the Prx/Srx antioxidanting system is observed.

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7. A method of screening for diseases related to cancer, to ageing, to neurodegenerative diseases and to neuromuscular diseases, which method is characterized in that it comprises, for evaluating the involvement of the Prx/Srx antioxidanting system:

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(1) bringing the cells of a biological sample into contact, *in vitro*, with hydrogen peroxide (H_2O_2),

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(2) detecting the Prx-Cys_P-SO₂H formed, between 1 hour and 4 hours after said bringing into contact according to step (1), and

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(3) establishing the ratio of the amounts of Prx-Cys_P-SO₂H and of Prx-Cys_P-SH, from 4 hours after said bringing into contact according to step (1).

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8. A method of screening for diseases related to cancer, to ageing, to neurodegenerative diseases and to neuromuscular diseases, which method is characterized in that it comprises genotyping of the sulfiredoxin, using the total RNA of a suitable biological sample, in particular blood cells, in accordance with the following steps:

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- (1) extracting the total RNA from said biological sample,
- 5 (2) preparing specific sulfiredoxin cDNA by amplification of the RNA using the following two primers:
GTCCCGCGGCCGGCGGCGACG (SEQ ID No. 11)
AGCAGGTGCCAAGGAGGCTG (SEQ ID No. 12),
10 these sequences being located, respectively, upstream and downstream of the human sulfiredoxin ORF (GenBank No. AAH47707),
- 15 (3) establishing its nucleotide sequence, and
(4) comparing with respect to a DNA sequence encoding an Srx protein, as defined above, derived from the same species as that of the biological sample to be analyzed.
- 20 9. A method of screening for diseases related to cancer, to ageing, to neurodegenerative diseases and to neuromuscular diseases, which method is characterized in that it comprises relative quantification, by any appropriate means, of the mRNA encoding sulfiredoxin from the total cDNA prepared from a human biological sample, by comparison with a reference sample.
- 25 10. The method as claimed in claim 9, characterized in that said quantification comprises:

- 30 (a1) preparing cDNA from the total RNA by reverse transcription with appropriate primers, and in particular random hexanucleotide primers;
- 35 (a2) amplifying said cDNA in the presence of the pair of primers:
GTCCCGCGGCCGGCGGCGACG (SEQ ID No. 11)
AGCAGGTGCCAAGGAGGCTG (SEQ ID No. 12),

in the presence of a fluorescent reporter,
and simultaneously or sequentially,

- 5 (a3) detecting the amount of the amplimer (or
amplicon) by measuring the fluorescent
signal.
- 10 11. The method as claimed in claim 10, characterized
in that the fluorescent reporter is selected from
the group consisting of agents that bind to
double-stranded DNA and fluorescent probes.
- 15 12. The method as claimed in claim 10 or claim 11,
characterized in that, when said fluorescent
reporter is a probe, it is preferably selected
from the group consisting of the probes defined by
the following sequences:
20 TTAATTGAATTCATGGGGCTGCGTGCAGGAGG (SEQ ID No. 13)
and
25 TTTCCTTTGCGGCCGCCTACTACTGCAAGTCTGGTGTGGATG (SEQ
ID No. 14).
- 30 13. A method of screening for diseases related to
cancer, to ageing, to neurodegenerative diseases
and to neuromuscular diseases, which method is
characterized in that it comprises:
35 - immunodetection of the Srx protein in a
biological sample to be tested, using an
antibody obtained by suitable immunization of
an animal with an Srx protein or the peptide
FXGCHR, with X = G or S, after separation of
total proteins by electrophoresis, then
- evaluation of the quality and of the amount of
said Srx protein compared with a control Srx
protein.
- 40 14. The use of the sequence coding for an Srx protein,
as defined in claims 1 to 3, for obtaining plants

whose abilities to withstand stress are significantly increased.

15. Host cells, characterized in that they are
5 transformed with a recombinant vector containing a sequence encoding an Srx protein, defined by a sequence selected from the group consisting of the sequences SEQ ID Nos. 1-3, 5, 6 and 8-10.
- 10 16. The host cell as claimed in claim 15, characterized in that it consists of an *S. cerevisiae* strain modified with a vector overexpressing the *SRX1* gene.
- 15 17. The host cell as claimed in claim 15, characterized in that it consists of a mammalian cell modified with a vector overexpressing the hSrx1 gene.
- 20 18. The host cell as claimed in any one of claims 15 to 17, characterized in that said vector is advantageously an *E. coli/S. cerevisiae* shuttle vector comprising, at an EcoRI cloning site, the sequence encoding the Srx protein and the promoter
25 of the Srx gene.
19. A method of screening for medicinal products capable of modulating the activity of the Prx/Srx antioxiidizing system, characterized in that it
30 comprises:
 - (1) bringing the substance to be screened into contact with host cells as claimed in any one of claims 15 to 18, in the presence of hydrogen peroxide,
 - (2) detecting the Prx-Cys_P-SO₂H formed, between 1 hour and 4 hours after said bringing into contact according to step (1),

- 5 (3) establishing the ratio of the amounts of
Prx-Cys_P-SO₂H and of Prx-Cys_P-SH, from 4 hours
after said bringing into contact according to
step (1).

10 20. A method of screening for medicinal products that
are useful in the treatment of cancers, of
neurodegenerative diseases and of neuromuscular
diseases, related to a fault in the Prx/Srx
antioxidizing system, characterized in that it
comprises:

15 a) bringing the substance to be tested into
contact with an extract of host cells as
claimed in any one of claims 15 to 18 or a
biological sample of a nonhuman transgenic
animal, in particular mice, selected from the
group consisting of animals in which the gene
of the Srx protein is knocked out and animals
in which the gene of the Srx protein is
overexpressed, in the presence of hydrogen
peroxide,

20 b) measuring, by any appropriate means, the
antioxidizing activity of the Prx/Srx system of
the mixture obtained in a), and

25 c) selecting the substances capable of stimulating
or of inhibiting said activity.

30 35 21. The method as claimed in claim 20, characterized
in that the measurement of said activity is in
particular carried out by detecting the Prx-
Cys_P-SO₂H formed, between 1 hour and 4 hours after
said bringing into contact according to step (a),
and establishing the ratio of the amounts of Prx-
Cys_P-SO₂H and of Prx-Cys_P-SH, from 4 hours after
said bringing into contact according to step (a).

22. A method of screening for medicinal products that are useful in the treatment of cancers, of neurodegenerative diseases and of neuromuscular diseases, related to a fault in the Prx/Srx 5 antioxidantizing system, characterized in that it comprises:
- (1) bringing the substance to be screened into contact with nonhuman transgenic mammals, in particular mice, selected from the group consisting of animals in which the gene of the Srx protein is knocked out and animals in which the gene of the Srx protein is overexpressed, and 10
- (2) measuring the survival of the animal.
23. Anti-Srx antibodies, characterized in that they 20 are obtained by suitable immunization of an animal with an Srx protein defined by a sequence selected from the group consisting of the sequences SEQ ID No. 1-3, 5, 6 and 8-10 or the peptide FXGCHR, with X = S, as claimed in claim 4.
24. A method of reducing a product comprising at least two cysteines with redox activity, which method is characterized in that it comprises bringing said protein into contact with a sulfiredoxin (Srx), as defined in claims 1 to 3, which comprises at least one catalytic site having the following motif: FXGCHR, with X = G or S, in the presence of ATP and of magnesium. 25
- 35 25. A method of synthesizing a product comprising Cys-SH residues from products comprising Cys-SO₂H residues, characterized in that it comprises a step consisting of reduction of the product comprising the Cys-SO₂H residues to a product

comprising Cys-SH residues, in the presence of a sulfiredoxin as defined in claims 1 to 3, of ATP and of magnesium.